



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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APR 28 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Triclopyr Herbicide - Review of Chronic Toxicity
and Carcinogenicity Studies in Rats and Mice (EPA
ID No. 464-EUP-OI/8G3571)

TOX Chem No.: 882I
MRID Nos.: 401077-01,
403566-01

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3-28-88

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3/31/88
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and

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Registrant: Dow Chemical Company
Midland, MI

Toxicology Branch (TB) has reviewed two major studies
submitted by the registrant in support of temporary tolerances
of triclopyr for rice. The studies were entitled:

- A. Triclopyr: 2-Year Dietary Chronic Toxicity-Oncogenicity
Study in Fisher-344 Rats, and
- B. Triclopyr: 22-Month Oral Chronic Toxicity and
Oncogenicity Study in Mice.

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Briefly, the conduct of each study, the results, and conclusions were as follows:

- A. 2-Year Dietary Chronic Toxicity-Oncogenicity Study in Fischer-344 Rats: Male and female Fischer-344 rats were fed diets containing triclopyr at dose levels of 0, 3, 12, or 36 mg/kg/day for 2 years. For the main study, 50 rats were used/sex/dose level while for the 6- and 12-month interim sacrifices, 10 rats were used/sex/dose level. Results reported here indicate that triclopyr does not cause any major toxic effects to male or female rats at the dose levels tested. Thus, for the chronic toxicity portion of the study, it does not appear that an effect level was established and that the NOEL was higher than the HDT (36 mg/kg/day). The only lesion that appeared to be dose-related was the increased pigmentation in the proximal descending tubules of female rats. However, based on the fact that the nature of this lesion was not established, only very slight to slight incidence of this lesion was seen, and the fact that the kidneys bearing this lesion were not associated with any other changes, the toxicological importance of this lesion could not be established.

Triclopyr does not appear to be oncogenic in male or female rats at the dose levels tested.

Due to a number of deficiencies listed below, TB gave a Core-Supplementary classification to both chronic toxicity and oncogenicity portions of the study. The study can be upgraded to a Core-Minimum classification pending a satisfactory resolution of the following issues:

1. Provide the Agency with data pertaining to triclopyr stability at room temperature at different time intervals and with data on triclopyr homogeneity in the diet (all dose levels).
2. Provide the Agency with a list of the clinical signs of toxicity, if any, for all groups with particular attention given to the urogenital tract and/or address effects arising therefrom.
3. Provide a justification for the use of two animals/cage throughout the study.

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4. Address the great variability observed in clinical chemistry individual values. (These results suggest loss of quality control.)
5. Carry out statistical analyses on all important data including clinical chemistry and organ weight data.
6. Address the major differences that exist between the 6-, 12-, and 24-month study groups particularly pertaining to the incidence of renal tubule pigmentation.
7. Establish the nature of the pigment in the renal tubules and supply the Agency with historical control data (same strain of rats from the same laboratory, for the period concurrent to this study and/or just prior to this study--1980 to 1986).

- B. 22-Month Oral Chronic Toxicity and Oncogenicity Study in Mice: Male and female ICR mice were fed diets containing 0, 50, 250, or 1250 ppm of triclopyr, for 95 weeks. For the main study, 60 mice were used/dose level/sex, while for the 6- and 12-month sacrifices, 10 mice were used/dose level/sex. Data presented here indicate that the HDT was sufficiently high to be accepted as the MTD in both sexes (based on depression of body weight gains). In general, treatment of male and female mice with triclopyr did not result in any toxic effects other than the approximately 10 percent depression in body weight gains in both sexes of the high-dose group. Thus, the LEL for systemic toxicity (depression of body weight gains) appears to be the HDT (1250 ppm) for both sexes. The NOEL is considered to be the MDT (250 ppm) in both sexes.

Triclopyr does not appear to be oncogenic in male or female mice at the dose levels tested.

This study was classified as Core-Supplementary due mainly to the fact that a great variability in organ weight values and in clinical and hematology parameter values was reported within groups. The sponsor should address this problem and, where possible, exclude outliers for each parameter and redo appropriate statistical comparisons. The study can be upgraded to Core-Minimum classification when this issue is resolved.

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Reviewed By: Y.M. Ioannou
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: A. B. Kocialski
Section VII, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Chronic Toxicity-Oncogenicity TOX Chem No.: 8821

MRID No.: 401077-01

Test Material: Triclopyr

Synonyms: Dowco 233

Study No.: K-042085-026

Sponsor: Dow Chemical Company

Testing Facility: Dow Chemical Company
Midland, MI

Title of Report: Triclopyr; 2-Year Dietary Chronic Toxicity-
Oncogenicity Study in Fischer-344 Rats

Authors: D.L. Gisenbrandt, H.M. Firchau, E.L. Wolfe, and T.D.
Landry

Report Issued: January 27, 1987

Conclusions:

The present study has investigated the chronic toxicity and oncogenicity of triclopyr (at dietary levels of 0, 3, 12, and 36 mg/kg/day) in male and female F344 rats. Data presented here did not allow for establishing conclusively the chronic toxicity and/or oncogenicity of triclopyr in rats. Additional data are required.

Classification:

Chronic Toxicity: Core-Supplementary

Oncogenicity: Core-Supplementary

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Materials and Methods:

The test material triclopyr (3,5,6-trichloro-2-pyridinyloxy acetic acid), used in this study was a white solid of a technical grade (acid form) with a purity of 98% or better. (Lot No.: AGR 204229). Male and female Fischer-344 rats (purchased from Charles River Breeding Laboratory, Kingston, New York) approximately 4 weeks old with an average weight of 97 g for females and 127 g for males were used in this study. Upon arrival, all animals were examined for health status and acclimated to laboratory conditions for 2 weeks. The rats used in the study were identified by a numbered ear tag and housed two per cage in animal rooms with a temperature of 22 °C, 40 to 60% relative humidity, and a 12-hour photocycle.

Study Design:

A total of 280 rats per sex were used for this study. The rats were allocated to four groups/sex based on their initial body weights as follows:

| Test Group | Dose(mg/kg) | Number of Animals/Group | | | | | |
|----------------|-------------|---------------------------|-------------------|-----------|---------------------------|-------------------|-----------|
| | | Male | | | Female | | |
| | | Main Study (24 Months) | Interim Sacrifice | | Main Study (24 Months) | Interim Sacrifice | |
| | | | 6 Months | 12 Months | | 6 Months | 12 Months |
| Control | 0 | 50 | 10 | 10 | 50 | 10 | 10 |
| Triclopyr(LDT) | 3 | 50 | 10 | 10 | 50 | 10 | 10 |
| Triclopyr(MDT) | 12 | 50 | 10 | 10 | 50 | 10 | 10 |
| Triclopyr(HDT) | 36 | 50 | 10 | 10 | 50 | 10 | 10 |

For the preparation of the test diets, triclopyr was dissolved in acetone and then mixed with the diet using a Hobart paddle mixer. This premix (0.25%) was then serially diluted until the desirable dose levels of 3, 12, and 36 mg/kg were obtained based on the body weight and food consumption data. Diets were prepared fresh every 2 weeks. Samples of the premix were analyzed for stability and homogeneity and test diets (all dose levels) were analyzed for triclopyr concentration.

All animals were observed twice daily for clinical symptoms of toxicity and mortality. Additionally, all animals were palpated for masses at the start of the study at 6 and 12 months and monthly thereafter. Body weights were recorded weekly for the first 13 weeks and monthly thereafter. Feed consumption was recorded at the same time intervals as body weight but only for subgroups of 20 rats/sex/dose.

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For hematology measurements blood was obtained by orbital-sinus bleeding from 20 rats/sex/dose for the main study and from 10 rats/sex/dose for the 6- and 12-month satellite groups at necropsy (animals anesthetized with methoxyflurane). The following CHECKED (X) parameters were measured:

| | | | |
|---|--------------------------|---|-----------------------------------|
| X | Hematocrit (HCT)* | X | Total plasma protein (TP) |
| X | Hemoglobin (HGB)* | X | Leukocyte differential count |
| X | Leukocyte count (WBC)* | | Mean corpuscular HGB (MCH) |
| X | Erythrocyte count (RBC)* | | Mean corpuscular HGB conc. (MCHC) |
| X | Platelet count* | | Mean corpuscular volume (MCV) |

For clinical chemistry measurements blood was collected at necropsy from the cervical blood vessels of 20 rats/sex/dose of the main study and all rats of the 6- and 12-month satellite groups. The following CHECKED (X) parameters were examined:

| | | | |
|---|---|---|----------------------|
| X | Electrolytes: | X | Other: |
| | Calcium* | X | Albumin* |
| | Chloride* | | Blood creatinine* |
| | Magnesium* | X | Blood urea nitrogen* |
| | Phosphorous* | | Cholesterol* |
| | Potassium* | X | Globulins |
| | Sodium* | X | Glucose |
| | Enzymes | | Total Bilirubin* |
| X | Alkaline phosphatase | X | Total Protein |
| | Cholinesterase | | Triglycerides |
| | Creatinine phosphokinase* | | |
| | Lactic acid dehydrogenase | | |
| X | Serum alanine aminotransferase (also SGPT)* | | |
| X | Serum aspartate aminotransferase (also SGOT)* | | |

For urinalysis, urine samples were obtained a few days prior to necropsy from 20 rats/sex/dose of the main study and 10 rats/sex/dose of the 6- and 12-month satellite groups. The CHECKED (X) parameters were examined:

| | | | |
|---|-------------------------|---|--------------|
| X | Appearance* | X | Glucose* |
| | Volume* | X | Ketones* |
| X | Specific gravity* | X | Bilirubin* |
| X | pH | X | Blood* |
| | Sediment (microscopic)* | | Nitrate |
| X | Protein* | X | Urobilinogen |

Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

| <u>X</u> | Digestive system | <u>X</u> | Cardiovasc./Hemat. | <u>X</u> | Neurologic |
|----------|------------------|----------|--------------------|----------|------------------------------------|
| X | Tongue | X | Aorta* | XX | Brain* |
| X | Salivary glands* | XX | Heart* | X | Periph. nerve* |
| X | Esophagus* | X | Bone marrow* | X | Spinal cord (3 levels) |
| X | Stomach* | X | Lymph nodes* | X | Pituitary* |
| | Jejunum* | X | Spleen* | X | Eyes (optic n.)* |
| | Ileum* | X | Thymus* | | Glandular |
| X | Cecum* | | Urogenital* | X | Adrenals* |
| X | Colon* | XX | Kidneys* | X | Lacrimal gland |
| X | Rectum* | X | Urinary bladder* | X | Mammary gland* |
| XX | Liver* | XX | Testes* | X | Parathyroid* |
| | Gallbladder* | X | Epididymides | X | Thyroids* |
| X | Pancreas* | X | Prostate | | Other |
| | Respiratory | X | Seminal vesicle | X | Bone* |
| X | Trachea* | X | Ovaries | X | Skeletal muscle* |
| X | Lung* | X | Uterus* | X | Skin |
| | | X | Cervix | X | All gross lesions and masses |
| | | X | Vagina | | |
| | | X | Oviducts | | |

*Recommended by Subdivision F (October, 1982) guidelines for chronic studies.

Statistical Evaluation (Abstracted from the original report): Statistical outliers were identified by a sequential test, but routinely excluded only from feed consumption statistics. Body weights were evaluated through 18 months on study by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, a parametric or nonparametric analysis of variance (ANOVA) was performed, followed respectively by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons.

The nominal alpha levels and references are as follows:

| | |
|---|-----------------------|
| Bartlett's test (Winer, 1971) | a= 0.01 |
| Parametric ANOVA (Hollander and Torrie, (1960) | a= 0.10 |
| Nonparametric ANOVA (Hollander and Wolfe, 1973) | a= 0.10 |
| Dunnett's test (Winer, 1971) | a= 0.05, two-sided |

Wilcoxon Rank-Sum test (Hollander and Wolfe, 1973)

a= 0.05,
two-sided

Bonferroni correction (Miller, 1966)
Outlier test (Grubbs, 1969)

a= 0.02,
two-sided

Descriptive statistics (mean and standard deviations) were calculated for feed consumption, body weights after 18 months on study, organ weights, clinical chemistry data, hematology data, and urinary specific gravity. Gross pathologic observations were tabulated and considered in the interpretation of final histopathologic data but were not evaluated statistically.

The cumulative incidence of appropriate histopathologic observations on all animals was used in statistical analysis. When a tissue was examined from all animals in all dose groups, the incidences of specific observations were tested first for deviation from linearity by ordinal spacings of the doses. If linearity was not rejected, the data were then tested for a dose-response relationship using the Cochran-Armitage Trend test. If the trend was statistically significant, or if significant deviation from linearity was found, the incidences for each dose group were compared to that of the control group using a pairwise chi-square test with Yate's continuity correction.

When a tissue was examined from all control and high-dose rats, but only from selected rats in the intermediate-dose groups, statistical analyses were limited to comparisons of control and high-dose observations. A pairwise chi-square test was used with Yate's continuity correction.

Differences in mortality patterns were tested by the Gehan-Wilcoxon procedure.

The nominal alpha levels are as follows:

| | |
|---|-----------------------|
| Chi-square test for lack of linearity (Armitage, 1971) | a= 0.01 |
| Trend test (Armitage, 1971) | a= 0.02, two sided |
| Pairwise comparison test (Fleiss, 1981) | a=0.05, two sided |
| Gehan-Wilcoxon test (Breslow, 1970) | a= 0.05 |

Because numerous measurements on the same set of animals were statistically compared, the frequency of false-positive (Type I) errors was unknown, but would be greater than the nominal alpha level. The final toxicological interpretation of the data considered other factors, such as dose-response relationships, biological plausibility and consistency, and historical rates.

Results:

The authors reported that triclopyr was stable in the diet for at least 8 weeks. However, no data on triclopyr stability were presented and no details were given as to the conditions (mainly temperature) under which these tests were carried out. Similarly, the authors reported that triclopyr was homogeneously mixed in the diet but no data were presented. Triclopyr concentrations in the diet were analyzed for all dose groups 10 times throughout the course of this study. Data reported here indicate that, for the most part, target concentrations were comparable to nominal concentrations. For the high dose tested (36 mg/kg/day) in male rats the feed concentration was 21% higher than the target concentration on week 69 of analysis. On weeks 28 (male rats) and 95 (female rats) diet concentrations for the low dose were 13% lower than the target concentration of 3 mg/kg.

Clinical Signs: The authors did not report any clinical signs of toxicity for these animals during the study.

Mortality: As shown below, the mortality at study termination was lower in the treated groups of male rats compared to controls (50, 32, 26, and 36% for control, low, mid, and high dose groups, respectively), and slightly lower than controls in female treated rats (20, 14, 18, and 18% for control, low, mid, and high dose groups, respectively).

Cumulative Mortality (%) - Main Study

| Month on Study | Males: Dose (mg/kg) | | | | Females: Dose (mg/kg) | | | |
|----------------|---------------------|----|----|----|-----------------------|----|----|----|
| | 0 | 3 | 12 | 36 | 0 | 3 | 12 | 36 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 |
| 15 | 0 | 4 | 0 | 0 | 2 | 0 | 2 | 0 |
| 18 | 4 | 6 | 2 | 2 | 2 | 0 | 2 | 2 |
| 21 | 20 | 12 | 4 | 12 | 14 | 2 | 8 | 4 |
| 24 | 50 | 32 | 26 | 36 | 20 | 14 | 18 | 18 |

Mean body weights were practically identical between treated and control groups in male rats throughout the study. In female rats, a statistically significant increase in body weight was seen in all treated groups as compared to controls at different time intervals (day 198 through day 534 on study). Food consumption was approximately similar between treated and control groups in both sexes throughout the study.

From the hematology parameters measured only a few were found to be statistically significantly different between the treated and control groups. As shown in Table 1, in male rats treated with the high-dose level (36 mg/kg/day), the erythrocyte count was numerically lower than controls at the 6- and 12-month sacrifice, and statistically significantly lower at the 12-month sacrifice. Hemoglobin content was statistically significantly lower than controls at the 6-month sacrifice and numerically lower at the 12- and 24-month sacrifice. Similarly, hematocrit was statistically significantly lower than controls at the 6- and 12-month sacrifice and numerically lower at the 24-month sacrifice. (Note: No statistical analysis of the 24-month hematology data was carried out by the authors.) In female rats all hematology parameters measured appeared to be similar between the treated and control groups at all time points examined.

The clinical chemistry parameters measured were approximately similar between the treated and control groups at the 6-month sacrifice in male and female rats and at the 12-month sacrifice in female rats. In male rats of the 12-month sacrifice urea nitrogen and albumin were statistically significantly lower in the high-dose group compared to controls. For the 24-month sacrifice, alanine aminotransferase activity and aspartate aminotransferase activity in male rats of the high-dose group appeared to be significantly higher than control values (no statistical comparisons of means were carried out by the authors).

None of the urinalysis parameters measured in male or female rats appeared to be significantly different between the treated and control groups in the satellite groups or the main study groups.

Absolute and relative organ to body weight ratios were for the most part similar between treated and control groups. However, the absolute and relative kidney weights of the high-dose group males of the 6- and 12-month satellite groups were found to be statistically significantly higher than control values. Numerically higher values in absolute and relative kidney weights were also seen with the main group males at all three dose levels tested with an apparent dose-related trend as shown in Table 2. (Note that no statistical comparisons of the means were carried out by the authors for the main groups.) Slightly higher absolute and relative liver weights were also seen in male rats of the main group at the mid- and high-dose levels tested.

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Table 1

Effect of Triclopyr on Selected Hematology Parameters - Male Rats

| Parameter | Time of Sampling | Dose (mg/kg/day) | | | |
|-------------------------------|------------------------|------------------|-------------|-------------|--------------|
| | | 0 | 3 | 12 | 36 |
| Erythrocytes (X10E6/cu mm) | 6 ¹ months | 9.04 + 0.24 | 8.84 + 0.36 | 8.86 + 0.23 | 8.81 + 0.33 |
| | 12 ² months | 9.03 + 0.22 | 9.09 + 0.19 | 9.02 + 0.16 | 8.81* + 0.19 |
| | 24 ³ months | 7.63 + 1.54 | 8.03 + 1.44 | 7.46 + 1.81 | 7.19 + 2.42 |
| Hemoglobin (G/DL) | 6 months | 16.5 + 0.6 | 16.0 + 0.6 | 16.0 + 0.4 | 15.7* + 0.5 |
| | 12 months | 14.3 + 0.4 | 14.2 + 0.1 | 14.2 + 0.2 | 13.9 + 0.3 |
| | 24 months | 13.2 + 2.3 | 13.2 + 2.2 | 13.2 + 2.5 | 12.5 + 3.6 |
| Hematocrit (%) | 6 months | 44.9 + 1.3 | 43.7 + 1.7 | 43.7 + 0.9 | 43.0* + 1.5 |
| | 12 months | 62.2 + 1.4 | 62.5 + 0.9 | 62.3 + 1.2 | 60.8* + 1.2 |
| | 24 months | 50.0 + 9.4 | 52.3 + 9.0 | 49.7 + 10.7 | 47.1 + 14.3 |

¹ 6-month interim sacrifice, N = 10² 12-month interim sacrifice, N = 10³ Final sacrifice, N = 20

* Statistically significantly different from controls; Dunnett's test; p = 0.05.

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Table 2

Male Rats: Effect of Triclopyr on Organ Weights

| Organ | Time of Sacrifice | A ¹ or R ² | Dose (mg/kg/day) | | | |
|--------|-------------------|----------------------------------|------------------|--------------|--------------|--------------|
| | | | 0 | 3 | 12 | 36 |
| Kidney | 6 months | A | 2.33 ± 0.16 | 2.26 ± 0.11 | 2.35 ± 0.17 | 2.57* ± 0.15 |
| | | R | 0.64 ± 0.03 | 0.64 ± 0.03 | 0.66 ± 0.04 | 0.71* ± 0.03 |
| | 12 months | A | 2.66 ± 0.16 | 2.73 ± 0.15 | 2.80 ± 0.14 | 3.11* ± 0.18 |
| | | R | 0.67 ± 0.03 | 0.67 ± 0.03 | 0.69 ± 0.02 | 0.75* ± 0.03 |
| | 24 months | A | 2.02 ± 0.24 | 3.18 ± 0.25 | 3.29 ± 0.31 | 3.33 ± 0.28 |
| | | R | 0.60 ± 0.07 | 0.84 ± 0.09 | 0.89 ± 0.15 | 0.90 ± 0.14 |
| Liver | 24 months | A | 12.76 ± 2.59 | 12.41 ± 2.41 | 13.04 ± 3.41 | 12.98 ± 1.93 |
| | | R | 3.38 ± 0.73 | 3.25 ± 0.62 | 3.51 ± 1.05 | 3.51 ± 0.73 |

1 Absolute organ weight (g)

2 Relative organ weight (g/100)

* Statistically significantly different from controls; Dunnett's test; p = 0.05

Gross pathology performed on all male and female rats that died spontaneously, killed in moribund condition or sacrificed at the end of the study (6 and 12 months for satellite groups, 24 months for main group) did not reveal any significant difference in the incidence of macroscopic lesions between the triclopyr treated and the control groups.

Histopathologic examination revealed a variety of microscopic lesions in several tissues of male and female rats mainly at the highest dose tested in the main study as well as the satellite groups. A summary of the most important lesions seen appears in Table 3. In the kidneys of the female rats (main study) there was a statistically significant increase in the incidence of pigmentation of the proximal descending tubules in all treated groups, compared to controls, with an apparent dose-related trend. Similar increase in proximal tubule pigmentation was also observed in the high-dose group females of the 6-month satellite group and the high-dose group males of the 12-month satellite group. In the kidneys of the 6-month satellite group there was also an increase in the degeneration of the proximal tubule in the mid- and high-dose group males. The combined incidence of

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adenocarcinoma (4/50) and adenoma (1/50) of the mammary gland of female rats of the high-dose group (36 mg/kg/day) - main study - was found to be statistically significantly higher than the control group (0/50). The incidence of mesothelioma in the testes (main study) was increased in a dose-related fashion (0/50, 1/50, 2/50 and 3/50 for the control, low-, mid- and high-dose groups, respectively. Similarly, there was a numerical increase in the incidence of pheochromocytoma of the adrenal in all treated groups of male rats and in the low- and high-dose groups of the female rats. Numerical increase in the incidence of skin papilloma and fibroma was also seen in the male rats.

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Table 3

Summary of Histopathological Observations

| Histopathological Observation | Males | | | | Females | | | |
|--|--------------------|-------|-------|-------|------------------|--------|--------|--------|
| | Dose (mg/kg/day) | | | | Dose (mg/kg/day) | | | |
| | 0 | 3 | 12 | 36 | 0 | 3 | 12 | 36 |
| <u>Main Study (24-Month Sacrifice)</u> | | | | | | | | |
| <u>Kidney</u> - Increased pigment proximal descending tubule - Very slight | 0/50 ^{1/} | 0/50 | 0/50 | 0/50 | 14/50 | 12/50 | 19/50 | 12/50 |
| - Slight | 0/50 | 0/50 | 0/50 | 0/50 | 1/50 | 17*/50 | 19*/50 | 29*/50 |
| Combined | 0/50 | 0/50 | 0/50 | 0/50 | 15/50 | 29*/50 | 38*/50 | 41*/50 |
| <u>Mammary Gland</u> - Adenocarcinoma | 0/50 | 1/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 4/50 |
| - Adenoma | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 1/50 |
| Combined | 0/50 | 1/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 5/50 |
| <u>Testes</u> - Mesothelioma | 0/50 | 1/50 | 2/50 | 3/50 | | | | |
| <u>Adrenals</u> - Pheochromocytoma, Benign (primary) | 6/50 | 13/50 | 17/50 | 11/50 | 1/50 | 6/50 | 1/50 | 5/50 |
| <u>Skin</u> - Papilloma - benign | 0/50 | 0/50 | 3/50 | 3/50 | 0/50 | 0/50 | 0/50 | 1/50 |
| - Fibroma, benign | 1/50 | 4/50 | 4/50 | 5/50 | 1/50 | 5/50 | 1/50 | 1/50 |
| <u>Satellite Group (6-Month Sacrifice)</u> | | | | | | | | |
| <u>Kidney</u> - Degeneration of proximal tubule, very slight and slight | 0/10 | 0/10 | 8/10 | 10/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| - Increased pigment-proximal tubule - Very slight | 0/10 | 0/10 | 0/10 | 0/10 | 1/10 | 0/10 | 1/10 | 9/10 |
| <u>Satellite Group (12-Month Sacrifice)</u> | | | | | | | | |
| <u>Kidney</u> - Increased pigment-proximal tubule - Very slight and slight | 0/10 | 0/10 | 4/10 | 10/10 | 0/10 | 0/10 | 0/10 | 0/10 |

^{1/} Number of rats with specified observation/total number of tissues examined

*Statistically identified difference from control group by Yate's chi-square pairwise test; p = 0.05

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Discussion

Triclopyr concentrations in the diet were with a few exceptions within acceptable limits from the target concentrations of 3, 12 and 36 mg/kg/day. The authors, however, failed to provide data on triclopyr stability in the diet at room temperature over a period of at least 24 hours. Similarly, although homogeneity tests were performed, according to the authors, no data were reported here. Thus, we request that the sponsor provide data on triclopyr stability and homogeneity in the diet.

The authors reported that no remarkable signs of toxicity were observed in any of the groups in this study. Again no data were provided to support this statement and therefore we request that such data be reported. Overall mortality was lower in female than in male rats and in both sexes, treated groups, had lower mortality than the control groups.

Mean final body weights in male rats were similar between treated and control groups. In female rats mean final body weights were usually higher (in many instances statistically significantly higher) in the treated groups compared to controls. Based on these results, it appears that the highest dose tested (36 mg/kg/day) was much lower than the desired maximum tolerated dose (MTD) for this study. The authors based the selection of the dose levels used for this study on a 13-week dietary study in Fischer-344 rats where the administration of 5, 20, 50, or 250 mg/kg/day of triclopyr resulted in:

- Decreased body weights in males treated with 20 or 250 mg/kg/day and in females treated with 250 mg/kg/day.
- Microscopic degeneration of renal proximal tubules in male and female rats treated with 20, 50, or 250 mg/kg/day.
- Increase in kidney weights in males treated with 50 or 250 mg/kg/day or in females treated with 250 mg/kg/day.
- Slight microscopic changes in liver cells in male rats of the high-dose group (250 mg/kg/day).

It is obvious that the authors considered the 20 mg/kg/day dose to be the MTD in the 13-week study, the toxic effect being the degeneration of renal proximal tubules. However, such an effect was seen only in the male rats at the 6-month sacrifice in the present study. None of the females at the 6-month sacrifice or males and females at the 12-month and 2-year sacrifice had this toxic effect. Thus, it seems that degeneration of renal proximal tubules is mostly a reversible effect apparently disappearing after 6 months of treatment. Based on the 13-week study, the mean body weights of male rats exposed to 20 mg/kg/day were statistically significantly lower than controls starting with the third week on

study and lasting until termination (89 days). On the contrary, in the present study (2-year study) no such decrease in body weights was seen with the high dose tested (36 mg/kg/day) at a comparable 90-day period in male rats. In fact, mean body weights were slightly higher than controls at the 90-day interval (2-year study) and body weights continued to increase throughout the study, reaching statistical significance by study termination. These discrepancies in the results between the 13-week study and the 2-year study make it difficult to say with certainty whether the MTD has been reached in this study. However, the available data from the 90-day study suggest that the selection of HDT in this study (36 mg/kg/day) was appropriate and can be accepted as the MTD.

Food consumption in male and female animals appeared to correlate with changes in body weights. Since two rats were used per cage, no individual animal data concerning food consumption could be obtained. The sponsor should justify why two rats per cage were used instead of one rat per cage.

The simultaneous decrease (in some cases statistically significant) in erythrocyte counts, hemoglobin content, and percent hematocrit in male rats of the high-dose groups at the 6-, 12- and 24-month sacrifice may be indicative of hemolytic anemia. However, in the present study this finding might not be of biological significance since there were no indications of morphologic alterations in the hematopoietic system of these animals.

A number of clinical chemistry parameters measured at the 24-month sacrifice appeared to be statistically significantly different between treated and control groups. However, these values are considered unacceptable in most cases due to the tremendous variation that exists between individual values within the same dose group. For example, the reported mean value for aspartate aminotransferase (AST) in the control group of male rats was 129 ± 97 (u/mL). The individual values for the same group ranged from 70 to 513 u/mL. For the same parameter (AST) the mean value reported in the high dose group, (36 mg/kg/day) was 247 ± 361 (u/mL) while the individual values (for 20 male rats) ranged from 52 to 1065 u/mL. Similar variations were seen with other parameters such as alanine aminotransferase activity and alkaline phosphatase activity. We consider this individual variation to be unacceptable.

The incidence of microscopic lesions in several tissues was higher in the treated groups as compared to control (Table 3). However, in most instances these lesions did not appear to be biologically significant mainly due to the lack of statistical significance, or a dose-related trend. Lesions that appeared to be of some importance were mainly the pigmentation of proximal descending tubules in the kidneys and the adenocarcinomas/adenomas in the mammary gland of female rats.

The increased pigmentation of the descending portion of proximal tubules in female rats was dose-related and statistically significantly higher in all treated groups (main study) compared to controls. However, the importance of this lesion is not evident mainly because: 1) only very slight to slight pigmentation was seen; 2) the nature of the pigment was not established by the authors, and 3) no other changes (morphologic or functional) were seen in the kidneys (for example, changes in absolute and relative kidney weight) of the affected animals. The interpretation of these results is further complicated by the fact that in female rats such lesions were present only in the high-dose group at the 6-month sacrifice and completely absent from all animals at the 12-month sacrifice. Although the authors stated in their report that the "pigment was present in virtually all male and female rats on the study, including controls" such was not the case since none of the male rats (control or treated) were reported to have this lesion. Thus, before the relative importance of this lesion can be assessed, the authors need to determine the nature of this pigment and also clarify statements made in the text of their report which are inconsistent with the reported results.

The following points can be made concerning the mammary gland tumors observed in female rats of the high dose group:

1. Adenocarcinomas were observed only in 4 out of 50 rats (8%) in the high dose group tested. No adenocarcinomas were seen in the control, low- or mid-dose groups.
2. Adenomas were observed only in 1 out of 50 rats (2%) in the high dose group and none in the control, low- and mid-dose groups.
3. The combined incidence of adenocarcinomas/adenomas in the high-dose group (5/50 or 10%) was statistically significantly higher than the concurrent control group (0/50 or 0%). However, there was no evidence of a dose-response (0/50 or 0% for low- and mid-dose groups) for these tumors. Furthermore, the incidence of these tumors in historical controls (see Appendix A, historical control data on mammary tumors in female F-344 rats; studies conducted just prior to the present study and at the same laboratory) was approximately 2.1% with a range of 0 to 4% for adenocarcinomas and 1.4% with a range of 0 to 4% for adenomas as compared to 0% for the concurrent controls. Additionally, the incidence of hyperplastic changes in the mammary gland was very low in the high-dose group, 3/50 or 6%.

Based on the aforementioned considerations, we do not consider the incidence of mammary gland tumors in female rats to be a strong evidence for classifying triclopyr as a carcinogen.

For a complete and final evaluation of this study, however, the sponsor should provide us with additional data and/or clarifications as follows:

1. Provide us with data pertaining to triclopyr stability at room temperature at different time intervals.
2. Provide us with data on triclopyr homogeneity in the diet (all dose levels).
3. Provide us with a list of the clinical signs of toxicity, if any, for all groups, with particular attention given to the urogenital tract and/or address effects arising therefrom.
4. Justify the use of two animals/cage, instead of the recommended one animal/cage.
5. Explain why there was such a great variability in clinical chemistry individual values. These results suggest breakdown in equipment, use of wrong procedures (loss of quality control), etc.
6. Carry out statistical analyses on all important data including clinical chemistry and organ weight data.
7. Address the major differences observed between the 6-, 12- and 24-month study groups particularly pertaining to the incidence of renal tubule pigmentation.
8. Establish the nature of the pigment in the renal tubules and supply us with historical control data (same strain of rats from the same laboratory, for the period concurrent to this study and/or just prior to this study 1980-1986).

Conclusions:

The present study has investigated the chronic toxicity and oncogenicity of triclopyr in male and female Fischer-344 rats, at dose levels of 3, 12 or 36 mg/kg/day. Data from the 90-day study suggest that the HDT (36 mg/kg/day) in this study can be accepted as the MTD. A number of significant deficiencies in this study, however, (see Discussion) made it difficult to conclusively establish the chronic toxicity and/or oncogenicity of triclopyr in male or female Fischer-344 rats.

Classification:

Chronic Toxicity: Core-Supplementary

Oncogenicity: Core-Supplementary

APPENDIX A

TRICLOPYR: 2-YEAR DIETARY CHRONIC TOXICITY/ONCOGENICITY
STUDY IN FISCHER 344 RATS
STUDY ID: K-042085-026

HISTORICAL CONTROL DATA FOR 2-YEAR STUDIES
MAMMALIAN AND ENVIRONMENTAL TOXICOLOGY RESEARCH LABORATORY

MAMMARY TUMORS IN FEMALE FISCHER 344 RATS
AS OF DECEMBER, 1986

| STUDY ^a (N) | ADENOMA | ADENOCARCINOMA |
|--|-------------|----------------|
| STUDY A - DIET CONTROLS (86) | 2 | 1 |
| STUDY B - INHALATION CONTROLS A (59) CONTROLS B (62) | 0 1 | 2 2 |
| STUDY C - DIET CONTROLS A (50) CONTROLS B (50) | 0 1 | 2 0 |
| STUDY D - DIET CONTROLS (50) | 2 | 0 |
| STUDY E - DIET CONTROLS (50) | 1 | 1 |
| STUDY F - INHALATION CONTROLS (50) | 0 | 1 |
| STUDY G - DRINKING WATER CONTROLS (60) | 0 | 2 |
| TOTALS CONTROLS (517) | 7 (1.4%) | 11 (2.1%) |

^a STUDIES WERE 24 MONTHS DURATION EXCEPT STUDY B WHICH WAS 27 MONTHS.

- DOW CONFIDENTIAL -

THE DOW CHEMICAL COMPANY
STUDY ID: K-042085-026
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Reviewed By: Y.M.. Ioannou
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: A.B. Kocialski
Section VII, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Chronic Toxicity and Carcinogenicity

TOX Chem No.: 882I

MRID No.: 403566-01

Test Material: Triclopyr

Synonyms: Dowco 233, Garlon

Study Number: Not Specified

Sponsor: Dow Chemical Japan, Ltd.

Testing Facility: Institute of Environmental Toxicology,
Tokyo, Japan

Title of Report: Triclopyr: 22-Month Oral Chronic Toxicity and
Oncogenicity Study in Mice

Authors: S. Tsuda, K. Ebino, M. Ikeda, T. Harada, and Y. Shirasu

Report Issued: April 1987

Conclusions:

The present study has investigated the chronic toxicity and oncogenicity of triclopyr at exposure levels of 0, 50, 250, or 1250 ppm for 95 weeks in male and female ICR mice. Data presented here indicate that triclopyr is not oncogenic at the dose levels tested. The LEL for chronic toxicity was tentatively considered to be the HDT (1250 ppm) while the NOEL was the MDT (250 ppm).

Classification: Core-Supplementary

Materials and Methods:

The test compound triclopyr (3,5,6-trichloro-2-pyridinyl-oxyacetic acid), a white solid with a purity of 98.0% (Batch No. AGR204229) was used in this study. Male and female specific-pathogen-free ICR mice (Jcl:ICR) purchased from Clea Japan, Inc., Tokyo, approximately 4 weeks old and weighing approximately a mean of 30.8 g (males) and 25.5 g (females) were used throughout this study. All animals were examined for health status during a 9- to 10-day acclimation period under laboratory conditions, and only healthy animals were used for the study. The animals were identified by markings on the head, back, or hip and housed in aluminum cages, four mice/cage and kept in air-conditioned rooms with a temperature of 24 ± 2 °C, humidity of $55 \pm 10\%$, 15 air changes per hour, and 14-hour light, 10-hour dark cycle. Feed (pulverized MF, feed M) and water were available to all animals ad libitum.

Study Design:

A total of 400 male and 400 female mice were used for this study. The mice were divided into four groups/sex based on their weight as follows:

| Group | Dose Level (ppm) | Male | | Female | |
|-----------------|---------------------|------------|-----------------|------------|-----------------|
| | | Main Group | Satellite Group | Main Group | Satellite Group |
| Control | 0 | 60 | 40* | 60 | 40* |
| Triclopyr (LDT) | 50 | 60 | 40 | 60 | 40 |
| Triclopyr (MDT) | 250 | 60 | 40 | 60 | 40 |
| Triclopyr (HDT) | 1250 | 60 | 40 | 60 | 40 |

*There were 10 mice sacrificed on week 26 and 10 on week 52 of the study. The remainder were sacrificed at termination of the study.

Test diets were prepared 2 to 3 times a week by mixing triclopyr with basal diet to obtain each desired dose level. The stability of triclopyr in the test diets was determined prior to this study using test diets from the 4-week preliminary feeding test (at concentrations of 200 and 800 ppm) either at 40 °C or at ambient temperature. The homogeneity of the test compound in the diet and test compound concentrations in the diet for all dose levels were determined at the beginning of this study and at approximately monthly intervals throughout the study.

Animals were observed daily for clinical signs of toxicity and mortality and palpated throughout the study for masses. Body weights were recorded weekly for the first 26 weeks on study and at biweekly intervals thereafter. Food consumption was measured twice a week for all groups using only eight cages/group for a total of 32 mice/group. Food efficiency was calculated using the body weight and food consumption data.

For hematological examinations blood was obtained from the posterior vena cava from 10 mice/dose/sex of the main group at the 95-week sacrifice and from 10 mice/sex/dose at the 26- and 52-week sacrifice (satellite groups). The following CHECKED (X) parameters were measured:

| | | | |
|----------|--------------------------|----------|-----------------------------------|
| <u>X</u> | | <u>X</u> | |
| X | Hematocrit (HCT)* | X | Total plasma protein (TP) |
| X | Hemoglobin (HGB)* | X | Leukocyte differential count |
| X | Leukocyte count (WBC)* | X | Mean corpuscular HGB (MCH) |
| X | Erythrocyte count (RBC)* | X | Mean corpuscular HGB conc. (MCHC) |
| X | Platelet count* | X | Mean corpuscular volume (MCV) |

For clinical chemistry determinations, blood collected from the same animals as mentioned in the hematology section was analyzed for the following CHECKED (X) parameters:

| | | | |
|----------|---|----------|----------------------|
| <u>X</u> | | <u>X</u> | |
| X | Electrolytes: | X | Other: |
| | Calcium* | X | Albumin* |
| | Chloride* | | Blood creatinine* |
| | Magnesium* | X | Blood urea nitrogen* |
| | Phosphorous* | X | Cholesterol* |
| | Potassium* | | Globulins |
| | Sodium* | X | Glucose* |
| | Enzymes | | Total Bilirubin* |
| X | Alkaline phosphatase | X | Total Protein* |
| | Cholinesterase | | Triglycerides |
| | Creatinine phosphokinase* | | |
| | Lactic acid dehydrogenase | | |
| X | Serum alanine aminotransferase (also SGPT)* | | |
| X | Serum aspartate aminotransferase (also SGOT)* | | |

For urinalysis, urine was collected from 10 animals/sex/dose of the 26- and 52-week satellite groups and from 10 animals/sex/dose of the main study (95-week sacrifice). The following CHECKED (X) parameters were examined:

| | | | |
|---|-------------------------|---|--------------|
| X | Appearance* | X | Glucose* |
| | Volume* | X | Ketones* |
| X | Specific gravity* | | Bilirubin* |
| X | pH | X | Blood* |
| | Sediment (microscopic)* | | Nitrate |
| X | Protein* | X | Urobilinogen |

*Recommended by Subdivision F (October 1982) guidelines for chronic studies.

Sacrifice and Pathology:

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

| X | Digestive system | X | Cardiovasc./Hemat. | | Neurologic |
|----|------------------|----|--------------------|----|------------------------------|
| | Tongue | X | Aorta* | XX | Brain* |
| | Salivary glands* | XX | Heart* | X | Periph. nerve* |
| X | Esophagus* | | Bone marrow* | X | Spinal cord |
| X | Stomach* | X | Lymph nodes* | | (3 level) |
| X | Duodenum* | XX | Spleen* | XX | Pituitary* |
| X | Jejunum* | XX | Thymus* | X | Eyes (optic n.)* |
| X | Ileum* | | Urogenital | | Glandular |
| X | Cecum* | XX | Kidneys* | XX | Adrenals* |
| X | Colon* | X | Urinary bladder* | | Lacrimal gland |
| X | Rectum* | XX | Testes* | X | Mammary gland* |
| XX | Liver* | X | Epididymides | XX | Parathyroids* |
| X | Gallbladder* | X | Prostate | XX | Thyroids* |
| X | Pancreas* | X | Seminal vesicle | | Other |
| | Respiratory | XX | Ovaries | | Bone* |
| X | Trachea* | X | Uterus* | X | Skeletal muscle* |
| X | Lung* | X | Vagina | X | Skin |
| | | | | X | All gross lesions and masses |

*Recommended by Subdivision F (October 1982) guidelines for chronic studies.

Statistical Evaluation (Abstracted from the Original Report):

Body weights, food consumption, water consumption, hematology and blood biochemistry data, and absolute and relative organ weights were evaluated as follows: Equality of variances was evaluated by Bartlett's test. When group variances were

homogeneous, a parametric analysis of variance of a one-way layout type was conducted to determine if any statistical differences existed among groups. When the analysis of variance was significant, Dunnett's t-test was used to identify statistically significant differences between treatment groups and their corresponding controls provided that the numbers of animals were equal in all groups. The Scheffe's multiple comparison test was applied when the animal numbers were not equal. When the group variances were heterogeneous, the data were evaluated by Kruskal-Wallis nonparametric analysis of variance. If significant, Dunnett-type mean rank test was applied when the numbers of animals were equal in all groups. The Scheffe's-type mean rank test was applied when the numbers of animals were not equal. The nominal 'a' levels for statistical tests were as follows: Bartlett's Test, $\alpha = 0.05$; analysis of variance $\alpha = 0.05$.

Incidence of clinical signs, mortality, and gross and histological lesions were evaluated by Fisher's exact test.

Results of urinalysis were evaluated by Wilcoxon-Mann-Whitney two-sample test.

Results:

Data presented here indicate that triclopyr is stable for at least 14 days at either ambient temperature or at 40 °C, when mixed with animal feed. Triclopyr was homogeneously mixed in the diet and diet concentrations (for all three dose levels tested) were, with a few exceptions, approximately similar to the target concentrations of 50, 250, or 1250 ppm.

A number of clinical symptoms were reported in animals of the main study and the satellite groups in both sexes. However, these observations could not be attributed to triclopyr treatment since the incidence of these symptoms was equally distributed between the treated and control groups.

Mortality:

As shown below, there were no appreciable differences in the mortality rate between treated and control groups in male or female mice.

Cumulative Mortality-Main Study

| Week of Study | Dose (ppm) | | | | | | | |
|---------------|------------|-------|-------|-------|--------|-------|-------|-------|
| | Male | | | | Female | | | |
| | 0 | 50 | 250 | 1250 | 0 | 50 | 250 | 1250 |
| 26 | 2/60* | 1/60 | 0/60 | 2/60 | 3/60 | 2/60 | 2/60 | 4/59 |
| 52 | 13/60 | 9/60 | 17/60 | 11/60 | 14/60 | 12/60 | 15/60 | 15/59 |
| 78 | 29/60 | 27/60 | 35/60 | 25/60 | 29/60 | 28/60 | 31/60 | 29/59 |
| 95 | 39/60 | 38/60 | 41/60 | 42/60 | 36/60 | 37/60 | 38/60 | 40/59 |

*Number of mice killed in extremis or found dead/total number of mice per group.

Mean Body Weights for male and female mice were approximately the same between controls and low- and mid-dose groups. Male and female mice of the high-dose group had consistently lower mean body weights throughout the study as compared to controls. In male mice of the HDT depressed body weights were statistically significantly lower than controls in the initial 44 weeks on study. Thereafter, until termination (95 weeks) only numerical depression in mean body weight was seen. Mean body weight gains were approximately 10.1 and 10.6 percent lower in the HDT (1250 ppm) in males and females, respectively, compared to controls at study termination. Food consumption was approximately similar between treated and control groups in both sexes. Chemical intake (calculated from the food consumption for each dose group) was found to be slightly lower in the female mice (on a mg/kg/day basis) of all treatment groups. Food efficiency did not differ between control and treated groups in both sexes. Mean water consumption in male mice of the HDT was statistically significantly higher than controls from week 53 on study to termination. At some intervals, water consumption in male mice of the HDT was over 50 percent higher than controls. An average of 26 percent higher water consumption was reported for the high-dose group males throughout the study compared to controls. In females, mean water consumption was numerically higher in the HDT compared to controls, mainly after the first year on study. Overall, 20 percent higher water consumption was reported between the HDT and the control group throughout the study.

From the urinalysis parameters measured, the specific gravity was statistically significantly lower in male mice of high-dose groups at the 26-, 52-, and 95-week timepoints. A slight decrease in specific gravity was also reported in the high-dose female mice at the 52- and 95-week intervals. Protein was present in the urine of all animals of the satellite groups and main study tested. However, higher protein concentrations were found in the urine of male mice of the 250 ppm group (main study) and in the females of the mid- and high-dose groups in the 26-week satellite group and in the high-dose group of the 52-week satellite group.

Hematology parameters appeared to be similar between treated and control groups at all timepoints examined. Due primarily to great variations in individual values in some instances, there appears to be significant differences between treated and control groups. In no case, however, do these differences imply dose-related response.

Clinical Chemistry: values were for the most part comparable between the treated groups and controls at the three timepoints examined. As shown in table 1, statistically significantly higher values in alanine aminotransferase (SGPT), albumin, and BUN were observed in male mice of the high-dose group at the 26-week interim sacrifice. BUN was also higher in the mid-dose tested (250 ppm) at the same timepoint. Within the different treatment and control groups, however, there was a tremendous variability in individual values of certain clinical parameters thus making the comparison between treatment groups meaningless. Greater individual variation was invariably observed in the main study (95-week sacrifice) in both sexes (table 1).

Table 1

Effect of Triclopyr on Selected Clinical Chemistry Parameters

| Parameter | Sex | Time of Sampling | Dose (ppm) | | | |
|----------------|-----|------------------|-----------------|-----------------|------------------|------------------|
| | | | 0 | 50 | 250 | 1250 |
| BUN (mg/dL) | M | 26 weeks | 23.8 \pm 2.7 | 24.1 \pm 2.8 | 31.2 \pm 3.4** | 29.7 \pm 4.9** |
| | F | 52 weeks | 25.0 \pm 6.4 | 24.7 \pm 6.3 | 25.6 \pm 8.2 | 31.9 \pm 15.9 |
| Albumin (g/dL) | M | 26 weeks | 1.50 \pm 0.10 | 1.50 \pm 0.08 | 1.53 \pm 0.08 | 1.63 \pm 0.15* |
| SGPT (u/L) | M | 26 weeks | 11 \pm 3 | 12 \pm 5 | 13 \pm 3 | 16 \pm 5* |
| | F | 26 weeks | 9 \pm 3 | 9 \pm 2 | 13 \pm 11 | 17 \pm 19 |
| | M | 95 weeks | 18 \pm 24 | 19 \pm 22 | 19 \pm 9 | 20 \pm 26 |
| SGOT (u/L) | F | 26 weeks | 34 \pm 3 | 40 \pm 12 | 144 \pm 343 | 44 \pm 33 |
| | | 52 weeks | 101 \pm 180 | 35 \pm 8 | 79 \pm 122 | 37 \pm 12 |
| | | 95 weeks | 46 \pm 21 | 65 \pm 48 | 79 \pm 100 | 67 \pm 77 |
| Alkaline | M | 95 weeks | 93 \pm 23 | 167 \pm 226 | 103 \pm 33 | 134 \pm 151 |
| Phosph. (u/L) | F | 95 weeks | 150 \pm 62 | 146 \pm 61 | 212 \pm 284 | 128 \pm 64 |

* Significantly different from the control; p = 0.05.

** Significantly different from the control; p = 0.01.

Absolute and Relative Organ Weights were found, in some instances, to be statistically significantly different between triclopyr-treated and control groups. As shown in table 2, the relative liver weight in male mice of the high-dose group at the 26-week sacrifice was statistically significantly higher than controls while the absolute liver weight in the same group was numerically higher than controls in all treated groups. Statistically significantly higher absolute pituitary weights were seen in female mice of the high-dose group at the 26-week sacrifice and in kidney weights at the 26- and 52-week sacrifice. Numerical differences in absolute and relative weights between treated and control groups were seen in many other organs in male and female mice at different sacrifice timepoints. In most instances, however, these changes could not be attributed to the test chemical, and in many cases within groups, variation in organ weights was so high (especially at terminal sacrifice) that a meaningful interpretation of the results could not be made (table 2).

Gross Pathology was performed on all male and female mice that died spontaneously, killed in moribund condition, or sacrificed at the end of the study (26 and 52 weeks for satellite groups and 95 weeks for main group) revealed that the incidence of a number of gross lesions was statistically significantly different between the treated and control groups. A summary of these lesions is shown in table 3. The enlargement of the thymus in male mice was statistically significantly higher in the mid- and high-dose groups compared to controls, with an apparent dose-related trend. Similarly, a higher incidence of liver enlargement was observed in all treated groups compared to controls in male and female mice. In male mice the incidence of blotted fur in the abdominal region was statistically significantly higher than controls in the high-dose group while in females the incidence of blotted fur on the external genital region was higher in all treated groups (statistically significantly higher in the mid-dose group) compared to controls.

Histopathologic examination revealed a variety of neoplastic and non-neoplastic lesions in most tissues examined in the control and treated groups of male and female mice. The incidence of neoplastic and non-neoplastic lesions was not statistically significantly different between treated and control groups. However, the incidence of some lesions was found to be numerically higher in the treated groups compared to controls in both sexes. The major changes seen for all animals examined are presented

Table 2

Effect of Triclopyr on Organ Weights

| Organ | Sex | A ¹ or R ² | Time of Sacrifice | Dose (ppm) | | | |
|----------------|-----|----------------------------------|-------------------|-----------------|-----------------|-----------------|-----------------|
| | | | | 0 | 50 | 250 | 1250 |
| Liver (g) | M | A | 26 weeks | 2.36 ± 0.25 | 2.58 ± 0.32 | 2.56 ± 0.29 | 2.76 ± 0.41 |
| | M | R | 26 weeks | 5.07 ± 0.39 | 5.39 ± 0.61 | 5.28 ± 0.36 | 5.87 ± 0.39** |
| Pituitary (mg) | F | A | 26 weeks | 3.1 ± 0.4 | 3.2 ± 0.4 | 3.5 ± 0.7 | 3.9 ± 0.7* |
| | F | R | 26 weeks | 0.0080 ± 0.0011 | 0.0080 ± 0.0017 | 0.0080 ± 0.0016 | 0.0094 ± 0.0020 |
| | M | A | 95 weeks | 2.4 ± 0.4 | 2.5 ± 0.3 | 2.6 ± 0.4 | 2.6 ± 0.4 |
| Kidney (mg) | F | A | 26 weeks | 448 ± 35 | 446 ± 37 | 477 ± 51 | 512 ± 55** |
| | F | R | 26 weeks | 1.14 ± 0.10 | 1.09 ± 0.11 | 1.09 ± 0.10 | 1.25 ± 0.17 |
| | F | A | 52 weeks | 532 ± 52 | 468 ± 68* | 524 ± 34 | 618 ± 73** |
| | F | R | 52 weeks | 1.20 ± 0.22 | 1.09 ± 0.12 | 1.22 ± 0.23 | 1.37 ± 0.16 |
| | F | A | 95 weeks | 578 ± 91 | 558 ± 87 | 608 ± 75 | 637 ± 100 |
| | F | R | 95 weeks | 1.28 ± 0.29 | 1.31 ± 0.29 | 1.31 ± 0.23 | 1.45 ± 0.25 |
| Spleen (mg) | F | A | 52 weeks | 106 ± 25 | 144 ± 86 | 287 ± 482 | 130 ± 44 |
| | F | R | 52 weeks | 0.27 ± 0.06 | 0.34 ± 0.17 | 0.68 ± 1.17 | 0.32 ± 0.10 |
| | M | A | 95 weeks | 235 ± 224 | 243 ± 395 | 306 ± 445 | 173 ± 128 |
| | M | R | 95 weeks | 0.52 ± 0.52 | 0.53 ± 0.84 | 0.63 ± 0.90 | 0.38 ± 0.27 |
| | F | A | 95 weeks | 309 ± 223 | 378 ± 228 | 278 ± 228 | 203 ± 125 |
| | F | R | 95 weeks | 0.69 ± 0.49 | 0.90 ± 0.59 | 0.59 ± 0.45 | 0.48 ± 0.35 |

1/ Absolute organ weight.

2/ Relative organ weight.

* Significantly different from the control; p = 0.05.

** Significantly different from the control; p = 0.01.

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TABLE 3

Summary of Macroscopical Observations¹

| Macroscopical Observations | Sex | Dose (ppm) | | | |
|---|-----|-------------------|-------|--------|--------|
| | | 0 | 50 | 250 | 1250 |
| <u>Thymus</u> - Enlargement | M | 0/60 ² | 2/60 | 6/60* | 9/59** |
| Blotted fur on abdominal region | M | 1/60 | 3/60 | 1/60 | 7/59* |
| <u>Liver</u> - Enlargement | M | 2/60 | 4/60 | 9/60* | 5/59 |
| | F | 7/60 | 11/60 | 11/60 | 14/59 |
| <u>Urinary Bladder</u> - Distended with urine | M | 13/60 | 19/60 | 18/60 | 21/59 |
| Blotted fur on external genital region | F | 1/60 | 6/60 | 8/60* | 6/59 |
| Anemia | F | 2/60 | 6/60 | 9/60* | 5/59 |
| Lymph node (cervical) - Enlargement | F | 7/60 | 8/60 | 16/60* | 10/59 |
| <u>Kidney</u> - Pale in color | F | 14/60 | 16/60 | 24/60* | 16/59 |
| <u>Ovary</u> - Cysts | F | 7/60 | 9/60 | 15/60* | 7/59 |

¹/ All animals examined.

²/ Number of mice with specified observation/total number of tissues examined.

* Significantly different from the control; $p = 0.05$.

** Significantly different from the control; $p = 0.01$.

in table 4 for the record. The only neoplastic lesion of some importance in this study appears to be the mammary gland adenocarcinoma, which increased in a dose-related fashion in female mice (0/60, 1/60, 2/60, and 4/59 for the control, low, mid and high dose groups, respectively).

Table 4

Summary of Histopathological Observations¹

| Histopathological Observations | Male Doses (ppm) | | | | Female Doses (ppm) | | | |
|---|---------------------|-------|-------|-------|-----------------------|------|------|-------|
| | 0 | 50 | 250 | 1250 | 0 | 50 | 250 | 1250 |
| <u>Neoplastic</u> | | | | | | | | |
| <u>Liver</u> - Hepatocelr. adenoma | 4/60 ² | 3/60 | 2/60 | 6/59 | 1/60 | 2/60 | 0/60 | 3/59 |
| <u>Mammary gland</u> - Adenocarcinoma | 0/60 | 0/60 | 0/60 | 0/59 | 0/60 | 1/60 | 2/60 | 4/59 |
| <u>Non-neoplastic</u> | | | | | | | | |
| <u>Kidney</u> - Tubular atrophy | 9/60 | 9/60 | 11/60 | 14/59 | 9/60 | 5/60 | 4/60 | 10/59 |
| - Pelvic dilatation | 12/60 | 16/60 | 17/60 | 19/59 | 4/60 | 4/60 | 1/60 | 6/59 |
| <u>Urinary Bladder</u> - Luminal dilatations | 13/60 | 19/60 | 18/60 | 21/59 | 0/60 | 1/60 | 0/60 | 0/59 |
| <u>Urethra</u> - Hemorrhage of urethral bulb | 5/60 | 7/60 | 7/60 | 11/59 | 0/60 | 0/60 | 0/60 | 0/59 |
| <u>Uterine Horn</u> - Luminal dilatation | 2/60 | 2/60 | 7/60 | 7/59 | - | - | - | - |

¹/ All animals examined.²/ Number of mice with specified observation/total number of tissues examined.

Discussion:

Triclopyr was found to be stable for at least 14 days when mixed with the diet and exposed to either ambient temperature or 40 °C. Furthermore, triclopyr was homogeneously mixed in the diet and diet concentrations were found to be comparable to target concentrations. None of the clinical signs of toxicity and/or mortality could be attributed to the test chemical since the incidence of toxicity signs and the rate of mortality were approximately similar between the triclopyr-treated groups and the control groups in both sexes. The mean terminal body weights of the high-dose groups (male and female) were slightly depressed (by 4 to 5%) compared to the corresponding controls. Mean body weight gains, on the other hand, were approximately 10 percent lower in the high dose groups compared to controls at study termination. Based on this finding, the high dose tested appears to approximate the MTD for both sexes. The authors based the selection of the dose levels used for this study on a 28-day range-finding study whereby male and female mice were exposed to dietary levels of 0, 200, 400, 800, 1600, and 3200 ppm of triclopyr. Briefly, the results of this study, according to the authors, were as follows:

1. Mice exposed to 3200 ppm showed anemia and weak renal toxicity.
2. Mice exposed to 1600 and 3200 ppm showed an increase in alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase activities; enlargement of the liver with dark color; and centrilobular swelling and degeneration of hepatocytes even at the 800 ppm dose level. Liver cell necrosis was seen in mice treated with 3200 ppm triclopyr.

The dramatic increase in water consumption by the high-dose group of male and female mice, as compared to controls, might suggest a toxic effect of the test chemical. However, none of the other parameters measured would support this finding.

The lower urine specific gravity observed with the high-dose group males and the increase in urine protein in the high-dose females (at different time intervals) are usually indicative of renal damage. However, these findings could not be substantiated with any histopathological kidney changes. Although a number of clinical chemistry and hematology parameters were found to be statistically significantly different between treated and control groups in both sexes, the significance of these differences could not be assessed due to the unusually high variability in individual values within groups.

Statistically significantly different than control absolute and relative organ weights were seen in several organs of the high-dose groups in both sexes mainly with the 26-week satellite group. These changes were not seen in the 52-week satellite group or the main study. Additionally, the great variation in organ weights within animals of the same treatment group makes the results very difficult to interpret.

From the pathological lesions, enlargement of the thymus appears to be dose related in male mice reaching statistical significance at the MDT and HDT. The authors do not consider this effect treatment related mainly because mice having enlarged thymuses also had malignant lymphomas of the thymus. Although the incidence of malignant lymphomas in male mice was not different between the treated and control groups, it is not clear why none of the control animals with malignant lymphomas (11/60) had enlarged thymuses while practically all treated animals with malignant lymphomas also had enlarged thymuses. Other macroscopic lesions reported in male and female mice did not appear to be treatment related.

The only histopathological neoplastic lesion that appeared to be of significance in this study was the incidence of mammary gland adenocarcinomas in female mice. This incidence was 0/60, 1/60, 2/60, and 4/59 for the control, LDT, MDT, and HDT, respectively. Although the induction of these tumors appears to be dose related, no statistical significance was achieved even with the HDT. No historical control data concerning this lesion have been provided by the sponsor which could possibly assist us in evaluating this finding.

Conclusions:

The present study has investigated the chronic toxicity and carcinogenicity of triclopyr in male and female ICR mice at dose levels of 0, 50, 250, and 1250 ppm for 95 weeks. Data presented have indicated that the HDT (1250 ppm) can be accepted as the MTD in both sexes (based on depression of body weight gains). However, parameters that can ordinarily be used to support toxicity endpoints, such as clinical chemistry measurements and/or changes in organ weights, associated in both cases with histopathological changes, could not be seriously considered in this study due mainly to the fact that intragroup variation in these values was not within acceptable limits. For the chronic toxicity portion of the study, tentatively the LEL is considered to be the HDT (1250 ppm) based on the depression of body weight gains in both sexes. The NOEL is considered to be the MDT (250 ppm) in both sexes.

Triclopyr is not considered to be oncogenic in male or female mice at the HDT (1250 ppm).

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However, for a final evaluation of this study, the sponsor should provide us with the following additional information and/or clarifications:

1. Provide the Agency with the 28-day preliminary range-finding study conducted with male and female mice. (The authors presented only a brief summary of the results in this report.)
2. Explain why within groups there was a tremendous variation in body weight values, and clinical chemistry and hematology parameter values. The authors should consider, where feasible, to exclude the outliers for each parameter and redo necessary statistical comparisons with the new values.

Classification: Core-Supplementary (The study can be upgraded to a Core-Minimum classification when the aforementioned issues are resolved)

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R:10346:Ioannou:C.Disk:KENCO:3/2/88:DD:VO:rw